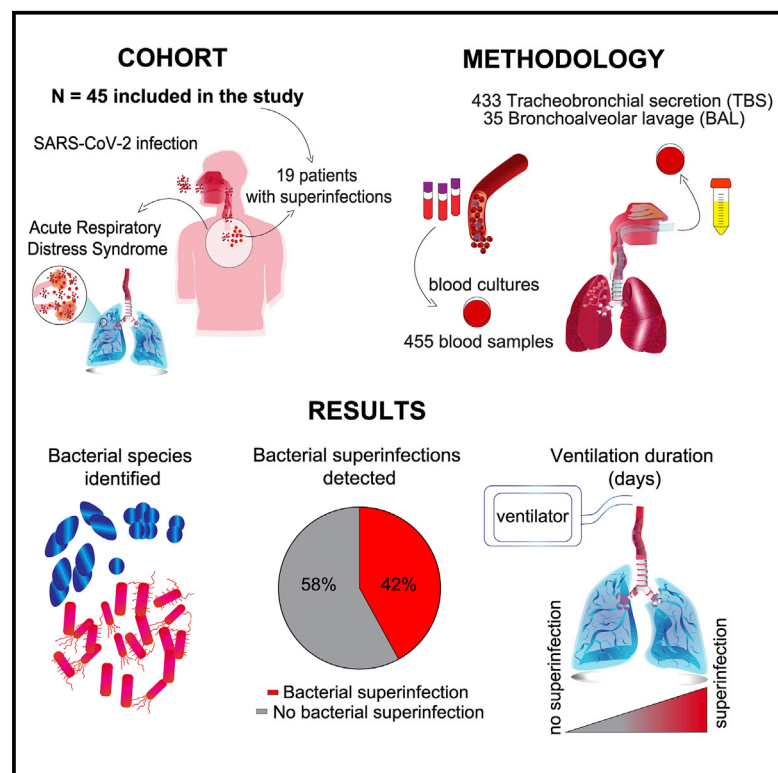


Bacterial pulmonary superinfections are associated with longer duration of ventilation in critically ill COVID-19 patients

Graphical abstract



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In brief

Buehler et al. show that detection of bacterial pulmonary superinfection is associated with a more severe disease course in COVID-19 patients, especially a lower likelihood of being alive and off invasive mechanical ventilation at study day 28 (ventilator-free days at 28 days).

Highlights

- Secondary bacterial infections (superinfections) are found in 42% of patients
- Bacterial superinfections occur on day 10 after intensive care admission
- Bacterial superinfections are associated with longer duration of ventilation
- Bacterial superinfections are mostly caused by Gram-negative bacteria

Report

Bacterial pulmonary superinfections are associated with longer duration of ventilation in critically ill COVID-19 patients

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SUMMARY

The impact of secondary bacterial infections (superinfections) in coronavirus disease 2019 (COVID-19) is not well understood. In this prospective, monocentric cohort study, we aim to investigate the impact of superinfections in COVID-19 patients with acute respiratory distress syndrome. Patients are assessed for concomitant microbial infections by longitudinal analysis of tracheobronchial secretions, bronchoalveolar lavages, and blood cultures. In 45 critically ill patients, we identify 19 patients with superinfections (42.2%). Superinfections are detected on day 10 after intensive care admission. The proportion of participants alive and off invasive mechanical ventilation at study day 28 (ventilator-free days [VFDs] at 28 days) is substantially lower in patients with superinfection (subhazard ratio 0.37; 95% confidence interval [CI] 0.15–0.90; $p = 0.028$). Patients with pulmonary superinfections have a higher incidence of bacteremia, virus reactivations, yeast colonization, and required intensive care treatment for a longer time. Superinfections are frequent and associated with reduced VFDs at 28 days despite a high rate of empirical antibiotic therapy.

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has evolved as the most relevant pandemic of modern history, challenging health care systems all over the world. The clinical characteristics of coronavirus disease 2019 (COVID-19) patients have been thoroughly described in recent studies.^{1–5} The triggers for acute respiratory distress syndrome (ARDS) in COVID-19 are virus initiated, subsequently leading to inflammation-mediated lung damage and endotheliitis.⁵ Although primarily a viral disease, antibiotics are empirically used in over 70% of cases in addition to experimental antiviral and immunomodulatory treatments.^{1,4–7} Secondary bacterial and/or fungal infections are a well-described phenomenon in viral illnesses, such as influenza, and are associated with increased morbidity and mortality in viral ARDS, as illustrated during previous pandemics.⁸ Secondary bacterial infections are typically referred to as superinfections, whereas co-infection is mainly used to describe simultaneous virus infection. Both co- and superinfections have been described in COVID-19 patients.^{6,9} Data regarding bacterial superinfections in COVID-19 pneumonia are limited and still emerging.^{10,11} A recent systematic review has concluded that the rate of bacterial/fungal superinfections is low,

arguing against the frequent use of broad-spectrum antimicrobials in patients with COVID-19.^{11,12} However, COVID-19-associated pulmonary aspergillosis (CAPA) has been reported in several cohorts of critically ill patients.^{13,14} Still, there is a lack of knowledge about the frequency and significance of bacterial, fungal, and viral concomitant infections in critically ill COVID-19 patients.¹² Additionally, in most studies performed so far, no thorough and systematic sampling for concomitant infections was performed. The high mortality in severely ill COVID-19 patients is thought to be at least in part due to secondary infections in addition to viral replication in the lower respiratory tract leading to severe lung injury and ARDS.^{6,15,16}

Superinfection seems to represent a major risk factor for mortality in COVID-19 patients.^{7,17–19} However, the risk of superinfection in mechanically ventilated patients with severe COVID-19 remains poorly described.

Currently, the diagnostic and treatment approach for superinfections remains unclear and the classical criteria for the detection of superinfections are often of limited use in COVID-19 patients. Clinical symptoms are an expression of the underlying disease of COVID-19 and cannot be used to reliably distinguish between patients presenting with or without relevant superinfections. For

Table 1. Baseline characteristics and study population

	Overall (N = 45)	No bacterial superinfection (n = 26)	Bacterial superinfection (n = 19)	p value
Baseline characteristics				
Age (years)	60 (54–69)	61.5 (54–71)	59 (54–69)	0.654
Male sex (n/%)	35 (77.8%)	19 (73.1%)	16 (84.2%)	0.375
Weight (kg)	83 (75–99)	80 (72.5–90)	92 (78–100)	0.049
Height (cm)	175 (165–182)	172 (160–180)	176 (169–185)	0.112
Body mass index (kg/m ²)	27.8 (25.7–31.6)	27.5 (25.6–30.6)	27.8 (26.8–35)	0.346
Comorbidity and other clinical conditions				
Myocardial infarction/ischemic heart disease	6 (13.3%)	5 (19.2%)	1 (5.3%)	0.222
Arterial hypertension	26 (57.8%)	16 (61.5%)	10 (52.6%)	0.550
Chronic kidney disease	12 (26.7%)	10 (38.5%)	2 (10.5%)	0.047
Dialysis	1 (2.2%)	1 (3.8%)	0 (0%)	0.999
Diabetes, no insulin therapy	5 (11.1%)	3 (11.5%)	2 (10.5%)	0.999
Diabetes, insulin therapy	14 (31.1%)	7 (26.9%)	7 (36.8%)	0.528
Asthma	1 (2.2%)	1 (3.8%)	0 (0%)	0.999
Chronic obstructive pulmonary disease (COPD)	5 (11.1%)	4 (15.4%)	1 (5.3%)	0.378
Renal or liver transplantation	5 (11.1%)	4 (15.4%)	1 (5.3%)	0.378
Immunosuppression	7 (15.6%)	5 (19.2%)	2 (10.5%)	0.222
Cancer	4 (8.9%)	2 (7.7%)	2 (10.5%)	0.999
Smoking	14 (31.1%)	7 (26.9%)	7 (36.8%)	0.625
Alcohol abuse	1 (2.2%)	0 (0%)	1 (5.3%)	0.999
Drug abuse	0 (0%)	0 (0%)	0 (0%)	1.000
Medical therapy before admission				
Statin	10 (22.7%)	6 (24%)	4 (21.1%)	0.999
Angiotensin-converting enzyme (ACE) inhibitor	7 (15.9%)	4 (16%)	3 (15.8%)	0.999
Scores/index				
Sepsis-related organ failure assessment score (SOFA)	8 (5–10)	8 (3–10)	9 (7–10)	0.480
Simplified acute physiology score (SAPS) II	36 (25–50)	32.5 (24–50)	42 (28–51)	0.275
Lowest PaO ₂ /FiO ₂ ratio at admission	122 (94–177)	129 (97–200)	108 (85–163.5)	0.228
Organ failure during ICU stay				
Acute kidney injury	27 (60%)	15 (57.7%)	12 (63.2%)	0.712
Dialysis	18 (40%)	9 (34.6%)	9 (47.4%)	0.388
Invasive mechanical ventilation	40 (88.9%)	21 (80.8%)	19 (100%)	0.043
Extracorporeal life support (ECLS)	8 (17.8%)	3 (11.5%)	5 (26.3%)	0.253
Rescue therapies				
Prone position	28 (62.2%)	11 (42.3%)	17 (89.5%)	0.001
Inhaled nitric oxide (iNO)	11 (24.4%)	5 (19.2%)	6 (31.6%)	0.341
Tracheotomy	20 (44.4%)	6 (23.1%)	14 (73.7%)	0.001
Timing (days)				
Time to ICU admission	2 (1–6)	2 (1–6)	3 (1–6)	0.636
Duration prone position	6 (1–10)	1.5 (1–7)	6 (4–10)	0.022
Duration iNO therapy	3 (1–6)	1 (1–3)	4.5 (1–22)	0.349
Duration intubation to tracheotomy	20 (10–33)	10.5 (9–21)	27 (15–33)	0.114

(Continued on next page)

Table 1. Continued

	Overall (N = 45)	No bacterial superinfection (n = 26)	Bacterial superinfection (n = 19)	p value
Laboratory values at admission				
White blood cell (WBC) count (G/L)	7.7 (5.7–10.7)	7.5 (5.7–10)	8 (5.3–13.4)	0.515
Hemoglobin (gr/L)	118.5 (101.5–133)	117 (107–132)	126 (98–134)	0.896
Hematocrit (%)	0.4 (0.3–0.4)	0.4 (0.3–0.4)	0.4 (0.3–0.4)	0.619
Platelet count (G/L)	199 (169.5–272)	200 (177–271)	190 (154–297)	0.776
Alanine transaminase (ALT) (U/L)	42.5 (25.5–65.5)	31 (24–60)	56 (33–72)	0.008
Lactate dehydrogenase (LDH) (U/L)	676 (527–842.5)	619 (471–742)	772 (626–876)	0.144
Alkaline phosphatase (U/L)	74.5 (53.5–103.5)	77.5 (56–108)	61 (53–98)	0.308
Urea (mmol/L)	6 (4.3–10.3)	6.2 (4.1–7.9)	6 (4.4–12.2)	0.651
Creatinine (μmol/L)	92.5 (67–138.5)	95 (70–128)	91 (57–149)	0.387
Serum sodium (mmol/L)	138 (134–141)	137 (135–140)	141 (134–146)	0.203
Serum potassium (mmol/L)	3.9 (3.7–4.5)	4.1 (3.7–4.4)	3.8 (3.5–4.7)	0.601
C-reactive protein (CRP) (mg/L)	168.5 (83.5–276.5)	124 (62–238)	255 (102–301)	0.034
Procalcitonin (PCT) (mcg/L)	0.3 (0.2–1.2)	0.2 (0.1–1.7)	0.4 (0.2–1.2)	0.060
Interleukin-6 (IL-6) (ng/L)	127 (71.2–454)	122 (84–697)	127 (62.7–263)	0.554
COVID-19 targeted drug therapy and antimicrobials				
Steroids	21 (46.7%)	8 (30.8%)	13 (68.4%)	0.012
Hydroxychloroquine	27 (61.4%)	13 (52%)	14 (73.7%)	0.143
Lopinavir/ritonavir	7 (15.9%)	4 (16%)	3 (15.8%)	0.999
Remdesivir	8 (18.2%)	4 (16%)	4 (21.1%)	0.704
Tocilizumab	2 (4.7%)	2 (8.3%)	0 (0%)	0.501
Empiric antimicrobial therapy	40 (8,839%)	22 (88%)	18 (94.7%)	0.441
Sample size overall				
TBS	433	114	319	
BAL	35	12	23	
Blood cultures	455	152	303	

Demographic and clinical characteristics as well as risk factors of COVID-19 patients stratified according to presence or absence of pulmonary relevant pathogens in tracheobronchial secretions (TBSs) and bronchioalveolar lavages (BALs) reflecting superinfection. The data are presented as median (interquartile range (IQR)) or number and percentage (%). The two groups were compared using chi-square test/Fisher exact or the Mann-Whitney test as appropriate.

this reason, several authors have argued in favor of an empirical antibiotic treatment with a focus on streptococci and staphylococci in severe courses.¹⁶ Other opinion leaders recommend (longitudinal) sampling of severely ill patients for early detection and treatment during the entire course of the disease.^{6,20}

Rapid diagnosis of co- and superinfections may not only help to improve survival but would also allow targeted antimicrobial therapy, improving antimicrobial stewardship throughout the course of the pandemic.^{20,21}

The aim of our study was to assess the burden of superinfections and the association with clinical outcomes in critically ill patients with COVID-19 ARDS (CARDS) in a tertiary care intensive care unit (ICU) with highly regulated antibiotic prescription.

RESULTS

Cohort characteristics

A total of 48 critically ill COVID-19 patients with ARDS were screened in the ICU at the University Hospital Zurich between April and June 2020. Three patients had to be excluded from the anal-

ysis because patients or relatives denied informed consent (Figure S1). 45 patients with a median age of 60 (54–69) years were included in this study. Most of them were male (35/45; 77.8%). Of the 45 patients, 19 (42.2%) were diagnosed with a superinfection. The median of ventilation duration was 15 days and length of ICU stay was 14 days overall. The median length of hospital stay was 24 days. Baseline characteristics are summarized in Table 1. In general, both groups of patients with and without superinfections were similar with regards to demographics and clinical characteristics. In particular, there were no differences in the severity of the disease and organ dysfunction as assessed by simplified acute physiology score (SAPS) II and sepsis-related organ failure assessment score (SOFA). Intensive care rescue therapies, such as prone position (42% versus 90%) and tracheotomy (23% versus 74%), were required more frequently and/or for longer periods in the superinfection group (Table 1).

Microbiological sampling and superinfections

Overall, 433 tracheobronchial secretions (TBSs), 35 bronchioalveolar lavages (BALs) samples, and 455 blood culture pairs

Table 2. Clinical outcomes and microorganisms detected

	No bacterial superinfection (n = 26)	Bacterial superinfection (n = 19)	p value
Outcomes and superinfection data			
Duration of ventilation (days)	8 (5.9–15.1)	37 (22.2–43.7)	<0.001
Length of ICU stay (days)	9 (7.0–14.9)	39 (28.5–57.0)	<0.001
Length of hospital stay (days)	17 (14.4–26.4)	44 (34.2–63.3)	<0.001
Patients died	6 (23.1%)	4 (21.1%)	0.999
Patients with bacterial respiratory superinfection	0 (0%)	19 (100%)	
Patients with superinfection detected in BAL	0 (0%)	6 (31.6%)	
Patients with bloodstream infection	2 (7.7%)	9 (47.4%)	0.004
Patients with <i>Aspergillus</i> detection	2 (7.7%)	3 (15.8%)	0.636
Patients with yeast colonization	12 (46.2%)	17 (89.5%)	0.004
Patients with multidrug resistant (MDR) pathogens	0 (0%)	10 (52.6%)	<0.001
Causative microorganisms			
Microbiology of superinfections			
Overall pathogen detection in TBS/BAL ^a	83	375	
Relevant pulmonary pathogen detection in TBS/BAL ^a	0 (0%)	169 (45.1%)	
<i>Citrobacter freundii</i> , <i>koseri</i>	0 (0%)	11 (2.9%)	
<i>Enterobacter cloacae</i>	0 (0%)	14 (3.7%)	
<i>Escherichia coli</i>	0 (0%)	2 (0.5%)	
<i>Klebsiella aerogenes</i>	0 (0%)	7 (1.9%)	
<i>Klebsiella pneumoniae</i>	0 (0%)	27 (7.2%)	
<i>Legionella pneumophila</i>	0 (0%)	2 (0.5%)	
<i>Pseudomonas aeruginosa</i>	0 (0%)	64 (17.1%)	
<i>Acinetobacter</i> spp.	0 (0%)	2 (0.5%)	
<i>Burkholderia cepacia</i>	0 (0%)	30 (8.0%)	
<i>Morganella morganii</i>	0 (0%)	3 (0.8%)	
<i>Streptococcus pneumoniae</i>	0 (0%)	2 (0.5%)	
<i>Streptococcus anginosus</i>	0 (0%)	3 (0.8%)	
<i>Staphylococcus aureus</i>	0 (0%)	2 (0.5%)	
Microbiology of bloodstream infections			
Overall	2/152 (1.3%)	12/303 (4.0%)	
<i>Citrobacter</i> spp.	0 (0%)	2 (16.7%)	
<i>Enterococcus faecalis</i> and <i>E. faecium</i>	1 (50%)	6 (50%)	
<i>Klebsiella aerogenes</i>	0 (0%)	1 (8.3%)	
<i>Klebsiella pneumoniae</i>	0 (0%)	1 (8.3%)	
<i>Moraxella</i> spp.	1 (50%)	0 (0%)	
<i>Candida glabrata</i>	0 (0%)	1 (8.3%)	
<i>Pseudomonas aeruginosa</i>	0 (0%)	1 (8.3%)	
Viral detection			
Overall	2	9	
Influenza A virus	0 (0%)	1 (11.1%)	
Influenza B virus	0 (0%)	0 (0%)	
Respiratory viruses, multiplex PCR ^b (n = 34)	0 (0%)	0 (0%)	

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Table 2. Continued

	No bacterial superinfection (n = 26)	Bacterial superinfection (n = 19)	p value
Herpes simplex virus type 1 (HSV-1) (PCR in blood)	0 (0%)	5 (55.5%)	
Herpes simplex virus type 2 (HSV-2) (PCR in blood)	0 (0%)	0 (0%)	
Human herpesvirus 6 (HHV-6) (PCR in blood)	0 (0%)	2 (22.2%)	
Cytomegalovirus (CMV) (PCR in blood)	1 (50%)	0 (0%)	
Epstein-Barr virus (EBV) (PCR in blood)	1 (50%)	1 (11.1%)	

The data are presented as median (95% confidence interval [CI] of median) or number and percentage (%). The two groups were compared using chi-square test/Fisher exact or the Mann-Whitney test as appropriate.

^aOverall detected microorganisms, including repetitive detection in the same patient

^bMultiplex PCR assay for respiratory syncytial virus (RSV) A/B; influenza A/B virus; adenovirus; coronaviruses 229E, HKU1, NL63, and OC43; human bocavirus; human metapneumovirus (hMPV); rhino/enterovirus; and parainfluenza virus 1-4

were analyzed for the presence of microorganisms (Figure S1). The range of respiratory samples per patient was 1–36 with a median of 6 per patient.

In nineteen patients (42.2%), at least one clinically relevant bacterium or fungus was detected in TBSs/BALs during the study period, whereas in 26 patients (57.8%), no relevant microorganisms were detected in TBSs/BALs.

A total of 342 TBSs/BALs were collected in the superinfection group, and 169 pulmonary relevant microorganisms were detected in these samples. Only in two cases, results did not match between BALs and TBSs. TBSs became negative for detected pathogens within a median of 12 days. Despite high frequency of positive TBSs, blood cultures showed only seven different bacterial species in 14 positive blood culture pairs (Table 2).

In the group without superinfections, 83/114 TBS samples showed growth but without recovery of clinically relevant lung pathogens. *Candida albicans* was the most frequently isolated non-relevant organism. In the 12 BAL samples, there was also no evidence of pulmonary relevant microorganisms. However, bacteremia was detected twice in a total of 152 blood culture pairs (Table 2).

The detection time points of pulmonary relevant and non-relevant microorganisms are depicted in Figures 1 and S2, respectively. On average, relevant pulmonary pathogens were detected on day 10 after ICU admission and reflect the hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) spectrum (Figure 1A). Non-relevant pulmonary pathogens²² were detected on average on day 3 post-ICU admission (Figure S2). The most frequently isolated bacteria per patient were *Enterococcus* spp. (15/45), *Enterobacter/Citrobacter* (8/45), and *Klebsiella* spp. (7/45). Additionally, *Streptococcus pneumoniae* (2/45), *Streptococcus anginosus* (3/45), *Escherichia coli* (2/45), *Enterobacter* spp. (6/45), *Citrobacter* spp. (3/45), *Pseudomonas aeruginosa* (6/45), *Burkholderia cepacia* (2/45), and coagulase-negative staphylococci (13/45) were found.

Empirical antimicrobial therapy was given to 40/45 (88.9%) patients, antifungal therapy to 10/45 (22.2%) patients, and antiviral therapy to treat concomitant viral infections to 9/45 (20%) patients. Figures 1B and 1C summarize the antibiotic treatment received by the patients.

In ten patients (22.2%), multi-drug-resistant (MDR) bacteria (*Pseudomonas aeruginosa*, *Enterobacter cloacae*, and *Burkholderia cepacia*) were detected.

Serum reactivation of herpes simplex type 1 and 2 (HSV-1 and -2) was detected in 5 out of 45 patients. Human herpes virus 6 (HHV-6) was detected twice, cytomegalovirus (CMV) reactivation occurred once, and Epstein-Barr virus (EBV) reactivation occurred twice. One patient had a co-infection with influenza A virus (Table 2).

Furthermore, colonizations with fungi were detected, and the isolated organisms included *Candida* spp. (29/45 patients), non-*Candida* yeast (21/45 patients), and *Aspergillus* spp. (5/45 patients). A detailed overview of relevant respiratory pathogens detected in TBSs/BALs and blood cultures is shown in Table 2. Colonizing microorganisms not considered as being relevant pulmonary pathogens are depicted in Figure S2.

Proportion of participants alive and off invasive mechanical ventilation at study day 28 (ventilator-free days at 28 days)

COVID-19 patients with pulmonary superinfections had substantially lower ventilator-free days (VFDs) at 28 days than those without superinfections (Figure 2), with a subhazard ratio of 0.37 (95% confidence interval 0.15–0.90; $p = 0.028$).

Secondary outcomes

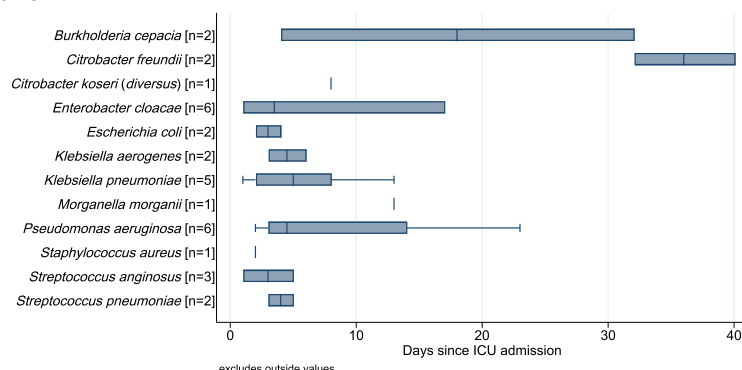
Patients with superinfections detected in respiratory specimens were ventilated for significantly longer time periods (8 versus 37 days; $p < 0.001$) and had a significantly longer duration of stay in the ICU (9 versus 39 days; $p < 0.001$) and overall hospitalization time (17 versus 44 days; $p < 0.001$) as compared to patients without superinfections (Table 2).

Further outcomes

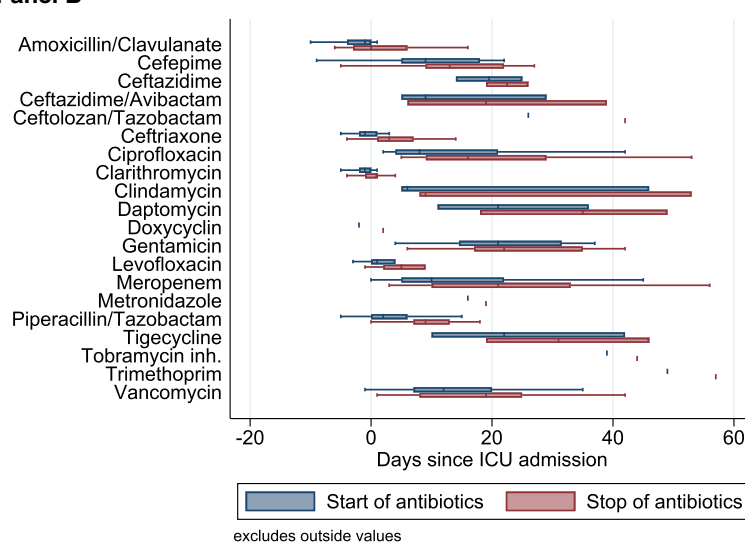
Patients with pulmonary superinfections had significantly more bacteremia ($p = 0.004$), virus co-infections/reactivations other than SARS-CoV-2 ($p = 0.001$), colonization with yeasts ($p = 0.004$), and infections with MDR pathogens ($p < 0.001$; Table 2).

Longitudinal laboratory inflammation parameters (leukocytes, C-reactive protein [CRP], procalcitonin [PCT], and neutrophil/lymphocyte ratio) for days 1–16 are shown in Figure S3 and

Panel A



Panel B



Panel C

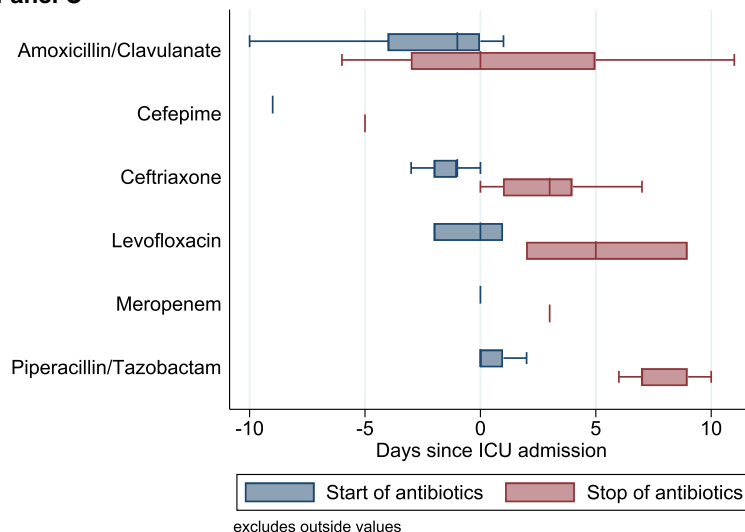


Figure 1. Spectrum and isolation time points in TBSs and BALs reflect hospital-acquired pneumonia (HAP)/ventilator-associated pneumonia (VAP) spectrum

(A) First detection time points of the most frequently cultured respiratory pathogens censored at 42 days (detection of *Acinetobacter baumannii* at day 77 and *A. bereziniae* at day 66). The n refers to the number of patients with a first detection of the respective pathogen. A total of 468 respiratory specimens from 45 patients were analyzed. Only the first detection event of a relevant respiratory pathogen in a given patient is reported.

(B) Antibiotics used for treatment of superinfections.

(C) Empiric antibiotic therapy used during first course of antibiotic treatment (before or at admission onto ICU).

See also Figures S1 and S2.

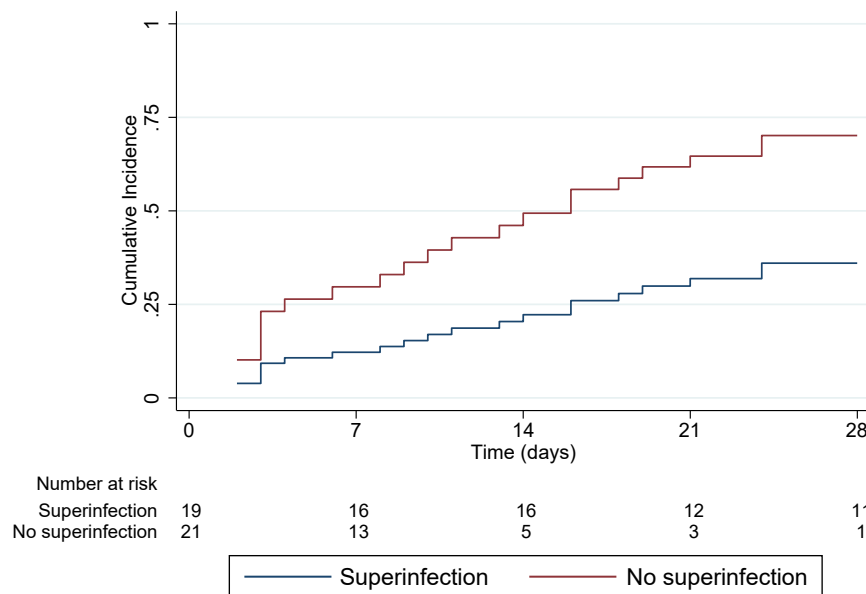


Figure 2. Proportion of participants alive and off invasive mechanical ventilation at study day 28 (ventilator-free days [VFDs] at 28 days)

Cumulative incidence curves for proportion of participants alive and off invasive mechanical ventilation at study day 28 (VFDs at 28 days). Only patients receiving invasive mechanical ventilation (N = 40) were included in this analysis. See also Figure S3 and Table S1.

Table S1. Only for CRP, the mixed-model evaluation showed a significant difference between the groups with increased CRP in the superinfection group ($p < 0.001$), whereas for leucocytes, there was a significant increase in numbers over time in both groups (Figure S3).

DISCUSSION

In this prospective cohort study of critically ill, ventilated COVID-19 patients, the presence of superinfection was associated with extended ventilation times, increased duration of intensive care and hospitalization, and increased need for intensive-care rescue therapies, such as proning and steroid use. Bacterial superinfections were detected in 42.2% of patients in our cohort, which is slightly higher than reported in previous studies.^{1,2,5,6,15,16,23} This discrepancy with other studies might be mainly due to the nature of our cohort consisting of severely ill patients with CARDS. In addition, differing from other studies, sampling was prospectively and repetitively scheduled and not only performed at admission as in other studies, which may account for underreporting of superinfections. Regional differences can also play an important role in bacterial superinfections and spectrum of resistance. This could explain in part the increased rate of superinfections compared to previous literature.^{11,24,25}

Although some studies have concluded that bacterial superinfections do not play a major role in disease severity and treatment choices, the results of the present study challenge the generalizability to severely ill CARDS patients.^{6,11} In our cohort, isolation of relevant respiratory bacteria was associated with more-severe COVID-19 disease courses with significantly longer duration of invasive mechanical ventilation and prolonged ICU and hospital stays. Compared to other studies investigating the role of superinfection in COVID-19, duration of ICU stay and length of ventilation was high, reflecting the disease severity of patients included in this study.^{7,11} Additionally, data on the

duration of ventilation and ICU stay are often missing in other studies, making comparisons difficult.^{4,11,15,21,26} Furthermore, due to the comparatively high SOFA score upon admission but moderate mortality, as in our cohort, long-term ICU-treatment complications, such as nosocomial infections, become more frequent.

Relevant respiratory bacteria were isolated on average on day 10 after ICU admission in our cohort, suggesting mainly nosocomial infections. In contrast to bacterial superinfections observed in influenza pneumonia, COVID-19 superinfections with Gram-positive bacteria, such as pneumococci or staphylococci, were rare in this study. It has to be mentioned that, in critically ill influenza patients, also infections with Gram-negative bacteria (such as *P. aeruginosa*) have been reported.²⁷ Similar observations were made for Middle East respiratory syndrome coronavirus (MERS-CoV)- and SARS-CoV-1-associated superinfections.^{28–30} In this study, mainly Gram-negative pathogens, such as *Pseudomonas* and Enterobacteriaceae, including MDR bacteria, were isolated, which is in line with previous reported studies.¹¹

Based on the findings that pulmonary bacterial superinfections seem to be mostly nosocomial and were associated with receipt of empiric broad-spectrum antibiotic therapy, use of antimicrobials may be more appropriately guided by the detection of pathogenic bacteria in longitudinal, high-quality respiratory samples. Patients could only be treated if pathogenic bacteria were detected in longitudinal samplings in line with antimicrobial stewardship interventions.^{12,26} Future prospective, randomized trials to investigate the efficacy of targeted antimicrobial therapy should be conducted to define best practice regarding prevention and treatment of bacterial superinfections in COVID-19. The isolation of mainly Gram-negative rods, including MDR, led to the use of third-line antibiotics, such as tigecycline, ceftazidime/avibactam, or ceftolozane/tazobactam, after initial empirical therapy of nosocomial pneumonia (Figure 1). It is important to consider the short- and long-term consequences that the use of antimicrobials, especially broad-spectrum, may have on drug resistance. A worrisome potential consequence of the COVID-19 pandemic might be the long-term spread of antimicrobial resistance (AMR) due to increased exposure of patients to antimicrobial agents that may have been used inappropriately.³¹ In this framework, employment of standardized longitudinal screening with early detection and

susceptibility testing before establishment of antimicrobial therapy could minimize the use of broad-spectrum, second- and third-line antibiotics, thus reducing AMR.

The high rate of yeast detection might be associated with the widespread use of broad-spectrum empirical antimicrobial therapy.³² Invasive aspergillosis was not detected by standard methods. However, molecular detection methods might be an important tool in future studies.^{33,34} So far, only few studies have investigated fungal superinfections in COVID-19 patients.^{13–15,17,35–37} The significance of viral reactivation remains unclear.¹¹ In our study, reactivations of HSV-1 and HHV-6 in the serum occurred in patients with bacterial superinfections. These findings support the hypothesis that superinfections associated with increased COVID-19 disease severity might enhance susceptibility to viral reactivations. Further studies with higher participant numbers should clarify the significance of this finding.

Prone positioning was more frequently performed in patients with superinfections. However, as patients received continuous subglottic suctioning, we believe that reflux during proning should not be an explanation for the observed superinfection differences. Rather, proning might reflect disease severity in the superinfection group. No selective digestive decontamination was performed.

In line with previous studies, conventional clinical laboratory tests, such as leukocytes, PCT, and neutrophil/lymphocyte ratio progressions, were not associated with pulmonary superinfections and therefore do not seem very useful for the detection of bacterial superinfections in COVID-19 patients on mechanical ventilation. Although CRP values differed significantly, the wide range observed in both groups did not allow the identification of a threshold value that clearly distinguishes patients with superinfection. This complicates the diagnosis of bacterial superinfections and emphasizes the importance of longitudinal microbiological diagnostics.

Advantages of this study are the prospective longitudinal monitoring of respiratory materials with concomitant recording of demographic data, microbiological evaluations, and antimicrobial therapy in a tertiary care center in a high-resource setting that did not experience health-care shortage during the first pandemic wave. Furthermore, this study used strict definitions for relevant respiratory pathogens. The diagnosis of superinfections was performed prospectively based on longitudinal sampling comprising detection of not only bacterial but also viral and fungal agents.

In summary, the detection of bacterial pulmonary superinfection was associated with a more-severe disease course in COVID-19 patients, especially a lower likelihood of being alive and off invasive mechanical ventilation at study day 28 (VFDs at 28 days). Future trials should investigate the effect of tailored antimicrobial therapy on outcome, antibiotic resistance, and drug use based on longitudinal assessment of respiratory tract cultures.

Limitations of study

Limitations of the study are the single-center design, small number of patients, and the high number of patients with empirical broad-spectrum antibiotic therapy (>90% of cases) at admis-

sion. Another limitation is the lack of a uniform, internationally valid definition of a bacterial infection of the lower respiratory tract and the inclusion of five patients that did not end up needing mechanical ventilation. However, patients not on mechanical ventilation were not included in the analysis of the primary outcome. Finally, as with all observational studies, causality cannot be inferred from reported associations.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at <https://doi.org/10.1016/j.xcrm.2021.100229>.

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AUTHOR CONTRIBUTIONS

Conceptualization, funding acquisition, and resources, P.K.B., A.S.Z., D.A.H., R.A.S., and S.D.B.; project administration, P.K.B. and S.D.B.; investigation, formal analysis, data curation, and visualization, P.K.B., P.D.W.G., D.A.H., P.M.F., S.M.S., A.G.-M., and S.D.B.; writing – original draft, P.K.B., D.A.H., S.D.B., P.M.F., and F.A.; writing – review and editing, P.K.B., A.S.Z., D.A.H., P.D.W.G., C.T.A., A.G.-M., S.M.S., F.A., M.A.M., J.B., M.P.H., P.M.F., R.A.S., and S.D.B. All authors revised the manuscript and approved the final version.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Tracheobronchial Secretions	University Hospital Zürich, Institute of Intensive Care Medicine	MicrobiotaCOVID biobank
Bronchoalveolar Lavages	University Hospital Zürich, Institute of Intensive Care Medicine	MicrobiotaCOVID biobank
Blood cultures	University Hospital Zürich, Institute of Intensive Care Medicine	MicrobiotaCOVID biobank
Software and algorithms		
STATA version 15	StataCorp, College Station, TX, USA	https://www.stata.com/stata15/
R version 3.6.3	R project	https://www.r-project.org/
SPSS Version 23	SPSS Science, Chicago, IL, USA	https://www.ibm.com/analytics/spss-statistics-software
Graphpad Prism 7	Graphpad, San Diego, CA, USA	https://www.graphpad.com/scientific-software/prism/

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contacts, Philipp K. Buehler (philipp.buehler@usz.ch) or Silvio D. Brugger (silvio.brugger@usz.ch).

Materials availability

This study did not generate new unique materials.

Data and code availability

The data and analysis scripts used in this study are available upon request to the Lead author.
No new code was generated.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Study design, ethics and population

The study was conducted as part of the MicrobiotaCOVID cohort study, a single-center, prospective observational study conducted at the Institute of Intensive Care Medicine of the University Hospital Zurich (Zurich, Switzerland) registered at clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT04410263). The study was approved by the Local Ethics Committee of the Canton of Zurich, Switzerland (Kantonale Ethikkommission Zurich BASEC ID 2020 - 00646).

Patients with confirmed SARS-CoV-2 infection and CARDS requiring ICU support and mainly invasive mechanical ventilation hospitalized between April 2020 and June 2020 during the first COVID-19 wave in Switzerland were eligible.

Inclusion criteria were age > 18 years, SARS-CoV-2 infection as determined by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) positivity of nasopharyngeal and/or pharyngeal swabs, TBS or BAL and hospitalization in the ICU for moderate or severe ARDS according to the Berlin criteria³⁸.

Exclusion criteria were patients or relatives denying informed consent and patients still being treated in the ICU when the study period ended.

Study outcomes

The primary outcome was proportion of participants alive and off invasive mechanical ventilation at study day 28, i.e., ventilator-free days (VFDs) at 28 days³⁹. Patients not on mechanical ventilation were excluded for the primary outcome analysis. Secondary outcomes were length of hospital stay, ICU stay and duration of mechanical ventilation. Further outcomes included the association

of pulmonary superinfection and bacteraemia, other virus co-infections/reactivations, colonization with yeast, bacterial infections with multidrug resistance (MDR), and longitudinal laboratory inflammation parameters.

METHOD DETAILS

Sample collection, processing and testing

If the respiratory situation, as assessed by the ICU attending physician in charge, allowed bronchoscopy, BAL (10ml of saline) was collected by the ICU personnel upon ICU admission and during the later course of the disease. TBS was collected from each ventilated patient at least on day 0 (i.e., upon ICU admission), day 1, day 2, day 3, day 5 and henceforth every 5 days. If the clinical situation did not allow TBS collection, no sampling was performed.

Samples were processed at the Institute for Medical Microbiology and at the Institute for Medical Virology of the University of Zurich. Standard clinical microbiology techniques were used for culturing, isolation and identification of bacterial and fungal microorganisms as previously described⁴⁰. SARS-CoV-2 was detected by real-time RT-PCR as previously described⁴¹. *Aspergillus* spp. was primarily identified in culture of TBS and BAL and also Galactomannan screening was performed in selected patients at physician's discretion.

At admission, multiplex PCR for respiratory syncytial virus (RSV) A/B, influenza A/B virus, adenovirus, coronaviruses 229E, HKU1, NL63 and OC43, human bocavirus, human metapneumovirus (hMPV), rhino/enterovirus and parainfluenzavirus 1-4 was performed in nasopharyngeal swabs. Multiplex PCR for the detection of atypical respiratory bacteria (*Legionella pneumophila*, *Chlamydia* spp., *Bordetella* spp. and *Mycoplasma* spp.) was performed on pharyngeal swabs at ICU admission.

Moreover, we assessed serum detection and viral load of the following viruses: herpesviruses type 1 and 2 (HSV-1 and -2), Cytomegalovirus (CMV), Epstein-Barr virus (EBV) and human herpesvirus 6 (HHV-6). Additional virus diagnostics, blood and urine cultures were initiated by the treating physicians according to the clinical situation.

Data collection and covariates

Clinical and laboratory data were obtained from electronic health records and included demographics, comorbidities / risk factors, medication, ICU scores, laboratory values, organ failure, need for invasive ventilation, need for extracorporeal life support (ECLS), rescue therapies, length of ICU/hospital stay, COVID-19 targeted experimental therapy (steroids, hydroxychloroquine, lopinavir/ritonavir, remdesivir, tocilizumab) and empiric antibiotic therapy.

Daily measurements of inflammatory parameters C-reactive protein and procalcitonin (CRP/PCT), leukocyte count and the neutrophil/lymphocyte ratio were routinely performed over the first 16 days after ICU admission.

Definition of superinfection

A multidisciplinary panel of ICU and infectious diseases consultants (unrelated to the study group) assessed the clinical status of the patients on a daily basis. Superinfection was diagnosed according to the panel's judgement of clinical deterioration and routine laboratory assessment as well as microbiological results. In more detail, the isolation of microorganisms from respiratory specimen cultures (TBS and/or BAL) regarded as clinically relevant by the panel was used as antimicrobial treatment guidance and the first specimen without pathogen growth was considered as the end of an episode in concordance with the clinical course. In case of isolation of more than one respiratory pathogen (i.e., in the bacterial and/or fungal analysis sample) in a given respiratory sample all were included in this study.

Organisms with low pathogenicity for lung infections such as *Enterococcus* spp., *Candida* spp., coagulase-negative staphylococci and non-pneumococcal streptococci were reported but not considered a relevant clinical pathogen of the airways in accordance with the literature⁴².

Detection of the HSV-1 and -2 as well as CMV, EBV, HHV-6 in blood were also reported but were considered reactivations. Viral co-infections/reactivations were only diagnosed if clinical signs of tracheitis or pathological signs of viral co-infection in cytology were observed.

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical analyses

Due to the unknown rate of concomitant infections in severely ill COVID-19 patients a power calculation was not feasible. Comparisons of population characteristics were performed using Mann-Whitney U tests and the Chi-square/Fisher exact test for categorical variables, as appropriate. For longitudinal analysis of laboratory parameters, differences between time points and superinfection status were tested using linear mixed effects models. To estimate the effect of superinfections on ventilator-free days (VFDs) at 28 days (proportion of participants alive and off invasive mechanical ventilation at study day 28), we used a competing risk regression model according to Fine & Gray censored at 28 days, with the event of extubation as outcome event and death as the competing risk. An alpha level of 0.05 was considered statistically significant. Statistical analysis was performed using STATA version 15 (StataCorp, College Station, TX), R version 3.6.3 (<http://www.r-project.org/>), SPSS Version 23 (SPSS Science, Chicago, IL, USA) and Graphpad Prism 7 (San Diego, CA, USA).

ADDITIONAL RESOURCES

This study has been registered at clinicaltrials.gov (ClinicalTrials.gov Identifier: NCT04410263, <https://clinicaltrials.gov/ct2/show/NCT04410263>). We followed the STROBE statement checklist in reports of cohort studies (<https://www.strobe-statement.org/index.php?id=available-checklists>).